

HOSTPLANT CHOICE OF CHECKERSPOT LARVAE:
EUPHYDRYAS CHALCEDONA, *E. COLON*, AND HYBRIDS
(LEPIDOPTERA: NYMPHALIDAE)*

BY M. DEANE Bowers

Museum of Comparative Zoology, Harvard University
Cambridge, Massachusetts 02138

INTRODUCTION

Hostplant preference and hostplant utilization abilities may vary among species, populations (Scriber, 1983; Blau and Feeny, 1983; Singer, 1982, 1983; Holdren and Ehrlich, 1982; Hsiao, 1978; Ehrlich and Murphy, 1981), and individuals (Rausher, 1978; Tabashnik, et al., 1981; Wasserman and Futuyma, 1981; Singer, 1982, 1983). Although such preferences and utilization abilities may be modified by environmental effects such as conditioning (Jermy, et al., 1964; Scriber, 1981; 1982; Grabstein and Scriber, 1982), there is clearly often an obvious genetic component to the patterns of hostplant use observed in nature (e.g., Jaenike and Grimaldi, 1983). The butterfly genus *Euphydryas* (Nymphalidae) is remarkable for the diverse strategies of hostplant exploitation exhibited by the six species that occur in North America. *Euphydryas gillettii*, for example, is reported to be virtually monophagous on *Lonicera involucrata* (Caprifoliaceae), while *E. editha*, *E. chalcedona*, and *E. anicia* are oligophagous, although individual populations may utilize a distinct subset of available hosts (Ehrlich, et al., 1975; Ehrlich and Murphy, 1981; Singer, 1982, 1983).

In butterflies, as in many other groups of insects, hostplant utilization is a function of oviposition preference of the female coupled with larval adaptation to the host. In the shift to a new hostplant, change in adult oviposition preference may occur more quickly than larval loss of the ability to utilize ancestral hostplants (Wiklund, 1975; Scriber and Feeny, 1979; Shapiro and Matsuda, 1980; Singer, 1982; Scriber, 1983). Thus adult oviposition behavior may not always reflect larval preference or fitness on a particular hostplant. In a series of elegant experiments with *Euphydryas editha*, Singer

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(1982, 1983) has shown that there is genetic variation in hostplant preference among individual females from a single population, as well as differences among females from different populations. Variation in the ability of *Euphydryas* larvae to use different hostplants has been investigated by several workers (Rausher, et al., 1981; Holdren and Ehrlich, 1982; Williams, et al, 1983a,b; Bowers, in prep.); however the genetic basis of larval preference in this genus has not been addressed.

The hostplants of the North American *Euphydryas* include primarily three families: Scrophulariaceae, Plantaginaceae, and Caprifoliaceae; with occasional use of plant species in the Oleaceae (Bowers, 1980); and Valerianaceae (Williams and Bowers, in prep.). These plants are all characterized by the presence of a group of secondary compounds, the iridoid glycosides (Kooiman, 1972; Jensen, et al., 1975; Bowers, 1981) which are used as larval feeding stimulants by *E. chalcedona* and probably by the other species as well (Bowers, 1983).

Neonate larvae potentially provide a powerful tool with which to examine the genetic basis of hostplant selection because their hostplant choice behavior is not complicated by the phenomenon of conditioning (Jermy, et al., 1964). Conditioning causes larvae to exhibit a preference for the plant species on which they have been feeding, and in some cases this may occur over a short period of time (Hanson, 1983). Hostplant choice of first instar larvae is usually considered to be constrained by the oviposition behavior of the female. However, Holdren and Ehrlich (1982) found that neonate larvae of *E. editha* and *E. anicia* may move as much as 15 cm in search of fresh food. *Euphydryas* hostplants include some species that are small annuals, and they may be defoliated or otherwise unsuitable as food, thus forcing even first instar larvae to move in search of other food. Ovipositing females may make mistakes (Chew, 1974), and although this may be rare in *Euphydryas*, in the laboratory, females with their feet on the hostplant, may lay their eggs on an inappropriate substrate (pers. obs.). The ability of newly hatched larvae to move in search of appropriate food is clearly important in the event of such a situation.

With these considerations in mind, I undertook a study of two species of *Euphydryas*, *E. colon* and *E. chalcedona*. These two species are very closely related (Bauer, 1975) and in the past have been

considered a single species (McDunnough, 1927; Gunder, 1929; dos Passos, 1964; Scott, 1980). The hostplants used by the *Euphydryas* populations that I studied are in different families: the *E. colon* population uses primarily *Symphoricarpos albus* in the Caprifoliaceae, while the *E. chalcedona* population feeds primarily on *Penstemon breviflorus* in the Scrophulariaceae. I compared the hostplant choice of neonate larvae of *E. chalcedona*, *E. colon*, and the hybrids.

MATERIALS AND METHODS

Euphydryas colon larvae were collected from a population at Satus Pass (ST) in Yakima County, Washington. In this population, larvae feed primarily on *Symphoricarpos albus* (Caprifoliaceae), although postdiapause larvae may occasionally be found feeding on *Penstemon* sp. (Scrophulariaceae). Both of these plant genera contain iridoid glycosides (Bowers, unpublished; Kooiman, 1970; Jensen, et al., 1974). *Penstemon* sp. and *S. albus* grow close to each other in this population.

Euphydryas chalcedona larvae were from a population north of Chico, on Crown Point Road (CPR), Butte County, California. The primary hostplant used in this population is *Penstemon breviflorus* (Scrophulariaceae). Oviposition and pre-diapause larval feeding are confined to this plant species; however post-diapause larvae may be found feeding on *Castilleja* sp. and/or *Diplacus bifidus* (Scrophulariaceae). These species all contain iridoid glycosides (Kooiman, 1970; Jensen, et al., 1975; Bowers, unpublished).

The post-diapause larvae collected from these populations, were brought back to Stanford University, and reared to the adult stage on leaves of *S. albus* (*E. colon*) or *P. breviflorus* (*E. chalcedona*). Adults were mated in net bags hung in a sunny window. For oviposition, mated females were put into glass cylinders covered with netting and containing a sprig of the appropriate hostplant. There appeared to be no problems in getting *E. colon* and *E. chalcedona* to hybridize in the laboratory, and a high proportion of the hybrid eggs were viable (Bowers, unpublished).

Egg masses were removed from the leaf on which they had been laid and kept in a growth chamber at 25 C Day:20 C Night, and a photoperiod of 16L:8D. When the larvae hatched, they were immediately given a choice test.

The choice tests were conducted in a small petri dish (6 cm diameter) lined with a piece of damp filter paper. Because *E. colon* and *E. chalcadona* are batch layers and the pre-diapause larvae are gregarious, the tests were conducted with groups of larvae. For each test, a group of 10 larvae was given a 5 mm disc of *P. breviflorus* and one of *S. albus*. These discs were punched out of the leaf with a cork borer, just prior to the beginning of each test. The discs were placed in the center of the petri dish about 1 mm apart, the larvae introduced, and allowed to feed for 48 hours. At 24 and 48 hours, the amounts of the two leaf discs eaten were estimated visually to the nearest ten percent (Jermy, et al., 1964; Bowers, 1979).

RESULTS

For each of the intraspecific crosses, the newly hatched larvae significantly preferred their own hostplant (Table 1, Fig. 1). In contrast, the hybrid larvae showed no preference for either plant (Table 1, Fig. 1). This ostensible lack of preference by the hybrid larvae, however, was not due to each group of larvae eating approximately equal amounts of both plant species, nor was it due to some larvae within a group feeding on the disc from one species and others on the other. On the contrary, each group of hybrid larvae appeared to

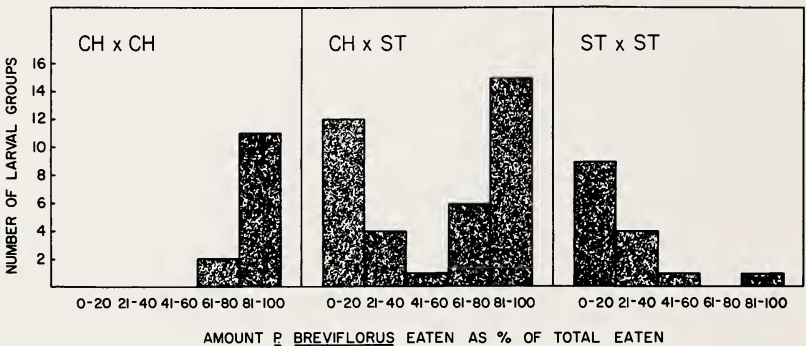


Fig. 1. The amount of *Penstemon breviflorus* eaten by *E. chalcadona* (CH × CH), *E. colon* (ST × ST) and the hybrids (CH × ST). The amount of *P. breviflorus* eaten is shown as the percent of the total amount eaten of both plant species.

Table 1. Comparison of the amounts eaten (percent) of *Penstemon breviflorus* and *Symphoricarpos albus* by *E. chalcidona*, *E. colon*, and the hybrids, after 24 and 48 hours. The data were analyzed by transforming the percentages using the arcsin transformation, and a one-way analysis of variance. Ch = Chico population of *E. chalcidona*, SP = Satus Pass population of *E. colon*.

Cross	♀ #	number of groups of 10 larvae	Mean amount eaten (%)					
			24 hours			48 hours		
			<i>P. brev.</i>	<i>S. albus</i>	p	<i>P. brev.</i>	<i>S. albus</i>	p
Ch × Ch	1(#15)	6	21.7	1.0	<.005	60.8	2.3	<.005
	2(#10)	4	10.0	1.8	<.025	65.0	4.5	<.005
	3(#14)	3	16.7	5.0	n.s.	40.0	10.3	<.05
	total	13	15.4	2.2	<.001	57.31	4.4	<.001
SP × Ch	4(#5)	18	9.3	6.1	n.s.	28.3	20.3	n.s.
	5(#2)	5	9.0	5.2	n.s.	44.0	19.0	n.s.
	6(#11)	6	6.8	9.3	n.s.	16.0	35.0	n.s.
	7(#12)	10	8.6	9.5	n.s.	32.5	14.5	n.s.
	total	39	8.7	7.1	n.s.	29.51	20.9	n.s.
SP × SP	8(#9)	15	4.6	7.9	<.01	10.7	44.3	<.005

choose one of the plants, most groups eating more *S. albus* or more *P. breviflorus* (Fig. 1). Of the 39 groups of hybrid larvae, chi-square analysis showed that nine groups of larvae significantly ($p < .05$) preferred *P. breviflorus*, five groups of larvae significantly preferred *S. albus*, and 25 groups of larvae showed no significant preference for either plant. As shown in Fig. 1, however, few of the groups ate equal amounts of both plant species. In virtually all of the choice tests the larvae clearly tasted both plant species, and thus were exercising a definite choice between the two discs.

To compare the amount of variation in hybrid offspring within and between different females, I compared the amount of *P. breviflorus* eaten as a percentage of the total amount eaten of both plant species. One-way Analysis of Variance using arcsin transformed percentages showed that there was more variation within the offspring of a single female, than among the four females (Table 2). Thus there was no genetic variation in hostplant preference among the hybrid offspring of the four females.

DISCUSSION

Larvae from the two populations of *E. chalcidona* and *E. colon* clearly showed a genetic preference for their own hostplant, while

Table 2. A. Amount of *Penstemon breviflorus* eaten by hybrid offspring as a percent of the total amount eaten of both plant species. Mean (S.D.) is given for each female. B. One-way analysis of variance comparing variation within the offspring of each female with that among individual females. Analysis performed with arcsin transformed data.

A.

	Female #			
	11	12	5	2
Percent <i>P. breviflorus</i> eaten	33.92 (32.07)	65.59 (28.45)	56.72 (39.05)	64.50 (38.13)
Number of groups of 10 larvae	6	10	18	5

B.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Among families	3	1955.28	651.76	.87	n.s.
Within families	35	26093.54	745.53		
Total	38	28048.81			

the hybrids were intermediate in their hostplant choice (Table 1). In general, pre-diapause larvae will attempt to feed on any plant that contains iridoid glycosides (Bowers, unpublished), although there may be differences in larval growth, survival, and digestive efficiency on hosts and non-hosts (Rausher, et al., 1982; Bowers, in prep.). Despite this general attraction of iridoid glycosides, purebred larvae in this experiment clearly chose the hostplant normally used in their population of origin. One interesting exception occurred in the offspring of the female from Satus Pass. One group of larvae ate only *P. breviflorus*, while all the others overwhelmingly preferred *S. albus* (Fig. 1). Post-diapause larvae at Satus Pass are found feeding on a *Penstemon* species, and this may be a factor in this result.

The feeding preference of the hybrid larvae overall was intermediate between that of the two parental species. Each group of ten larvae appeared to choose one of the leaf discs, but some groups chose *S. albus* and others chose *P. breviflorus*. Individual larvae were not tested, but hybrid individuals may prefer one or the other plant species, however, there appeared to be no division of the groups of larvae such that some individuals were feeding on one disc and some on the other—the larvae fed together on the same disc. As with many gregarious insect species, survival of individual *Euphydryas* larvae may be lower than that of groups, thus social facilitation or group effect is likely to be important in the feeding patterns actually observed. In these hybrid larvae, the genotype that is in the majority may guide the feeding of the rest of the group. Alternatively, all the larvae may be intermediate between the parental preferences and thus a choice is a function of factors other than genotype, such as position of the larvae.

The offspring of individual females all behaved similarly (Table 1): all purebred *E. chalcona* larvae preferred *P. breviflorus*, and although the offspring of only one *E. colon* female were tested, these larvae overwhelmingly preferred *S. albus*. Among the hybrid larvae, some groups preferred one plant species and others the other, while some showed no significant preference (Table 1, Fig. 1). There were no significant differences among the four females in the behavior of their offspring. Thus behavior of hybrid larvae did not differ from one female to the next. Although the offspring of only one *E. colon* female were tested, these larvae overwhelmingly preferred *S. albus* (Table 1). There was no significant effect of the female on the host-

plant choice of these hybrid larvae, indicating little or no genetic variation among individual females in their hostplant choice behavior. These results are in contrast with those of Tabashnik, et al. (1981), which showed significant genetic variation among individual females of *Colias eurytheme*, and Singer (1982) who found similar genetic variation in oviposition preference among individual females of *E. editha*.

Thus there is clearly a genetic component to the hostplant preferences exhibited by larvae of *Euphydryas*. However, the variation among individual females found by Tabashnik, et al. (1981) and Singer (1982) was not evident here, although the sample sizes were small. The preferences exhibited by the larvae are not absolute as larvae of both species did feed on the alternate food offered. These preferences may be important in the colonization of new population sites as well as in the incorporation of new hostplants into the existing repertoire of a particular population.

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